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EDITORIAL REVIEW

# Adaptation of the kidney to protein intake and to urine concentrating activity: Similar consequences in health and CRF

It has long been known that a high protein intake induces kidney hypertrophy and enhances renal hemodynamics. Because of the possible deleterious influence of dietary proteins in chronic renal failure, a number of investigations in the last decade have been devoted to the study of changes occurring in renal function (and morphology) after a protein meal or after chronic alterations in protein intake. Simultaneously, other studies have investigated the changes in renal function and morphology induced by chronic alterations in urinary concentrating activity and/or in circulating antidiuretic hormone (ADH) level.[1] It has become evident that these two conditions share a number of similar consequences including increased GFR and kidney weight with, in both cases, preferential enlargement of the renal zone and the nephron segment most directly involved in the process of urinary concentration.

This article reviews our present knowledge concerning these two adaptations and emphasizes the relationships between protein intake on the one hand, and urinary concentration or ADH on the other. A common mechanism is proposed to explain the similarity of the changes induced by the two conditions. This mechanism involves accumulation and recycling of urea in the renal medulla, which are known to be enhanced both by high protein intake and by ADH. Finally, the consequences of these protein/vasopressin-dependent effects on the progression of renal disease will be discussed.

## Adaptation of the kidney to urinary concentration or to protein intake: Experimental observations

### *Adaptation to urinary concentration and dilution*

Morphologic and functional changes in the kidney in response to changes in the level of urinary concentrating activity have been studied in the rat and a few other mammals by inducing chronic alterations in either the level of fluid intake or the level of ADH. Several studies have taken advantage of the Brattleboro rat, a strain in which the gene coding for ADH synthesis is defective and the hormone cannot be synthesized [1]. These rats suffer diabetes insipidus (DI), and the circulating ADH level can be easily manipulated by injections or chronic infusion of AVP or synthetic V2 agonists. In normal animals, water metabolism can be altered by reducing or increasing water intake, or by infusing AVP or synthetic V2 agonists in excess of the endogenous level [2, 3].

Until the last decade, studies on ADH action on the kidney had generally involved the *acute* effects of the hormone on its target cells and on the urinary concentrating process. Recently, several studies have revealed that *chronic* administration of vasopressin (in the form of its potent antidiuretic non-pressor V2 analogue, dDAVP [4]) or chronic stimulation of the urinary concentrating process leads to very specific adaptations of kidney function and morphology. These adaptations include: (1) an increase in whole kidney weight relative to body weight, with one of the renal zones, the inner stripe of the outer medulla, exhibiting greater hypertrophy than other zones; (2) an increase in GFR; (3) accentuation of the functional and anatomical heterogeneity between superficial and juxtamedullary nephrons; (4) specific hypertrophy and increase in transport capacity of the epithelium of the thick ascending limb of Henle's loop, restricted to its deepest part located in the IS; (5) increased reabsorption of calcium and magnesium in the thick ascending limb, leading to reduction of the urinary excretion of these two cations; (6) changes in medullary hemodynamics, in the production of some mediators, and in the chemical composition of the interstitium and epithelial cells.

*Renal hemodynamics and renal mass.* Chronic alterations in ADH level and in concentrating activity induce marked and parallel changes in kidney mass and hemodynamics. Brattleboro rats have smaller kidneys and a lower renal blood flow (RBF) than normal rats [5]. As shown in Table 1, both their kidney weight (relative to body weight) and their renal hemodynamics are markedly increased by chronic infusion of AVP or dDAVP [6–12]. Relative kidney weight and glomerular filtration rate (GFR) were also shown to vary in normal rat or mongolian gerbil as a result of sustained depression or stimulation of urine concentrating activity (Fig. 1 and Table 1) [2, 12, 13].

Figure 2 illustrates the influence of chronic vasopressin infusion on renal hemodynamics in conscious Brattleboro rats [9]. AVP increased GFR and renal blood flow (RBF) by 40 to 50%, an effect that develops slowly (no detectable change was observed after one hour AVP infusion in the same study) and regresses when hormone infusion is discontinued (Fig. 2). The influence of urinary concentrating activity on GFR is also apparent in normal rats. Four to seven days on an increased water intake, reducing urinary osmolality from 1,526 to 760 mOsm/kg $H_2O$, decreased mean daily GFR from 1.61 to 1.08 ml/min ($P < 0.001$). Conversely, chronic dDAVP infusion, raising urinary osmolality to 3,020 mOsm/kg $H_2O$, increased GFR to 1.89 ml/min [12] (Table 1). In conscious dogs, RBF was shown to increase significantly after 36 hours of water deprivation, when usual feeding was maintained [14].

That RBF and GFR might increase with increasing urinary concentrating activity and/or vasopressin level has long remained

[1] Rather than "vasopressin," the term "antidiuretic hormone" is often used in this review since the main effects considered here are dependent upon the antidiuretic and not the vasoactive actions of this hormone.

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**Table 1.** Influence of chronic alterations in water intake and/or ADH level, or in protein intake, on kidney weight and renal hemodynamics

| Ref. | Species and conditions: No or low UCA vs. high UCA | Kidney weight *mg/100 g BW*: No or low | High | High/low | Renal hemodynamics: Unit | No or low | High | High/low |
|---|---|---|---|---|---|---|---|---|
| Alterations in urinary concentrating activity (UCA) | | | | | | | | |
| 5 | Brattleboro DI rats vs. Wistar rats | 310 | 400 | 1.29 | RBF, ml/min × g KW | 2.18 ± 0.29 | 3.80 ± 0.51 | 1.62 |
| 6 | Brattleboro DI rats: Control vs. dDAVP injections | 329 ± 5 | 388 ± 18 | 1.18 | GFR, ml/min × 100 g BW | 0.46 ± 0.01 | 0.53 ± 0.05 | 1.15 |
| 7 | Brattleboro DI rats: Control vs. dDAVP infusions | 358 ± 13 | 441 ± 30 | 1.23 | SNGFR Superficial, nl/min | 34 ± 5 | 42 ± 6 | 1.24 |
| | | | | | SNGFR Juxtamed, nl/min | 31 ± 5 | 59 ± 7 | 1.90 |
| 9 | Brattleboro DI rats before vs. after chronic AVP infusion | — | — | — | RPF, arbitrary unit | 100 | 150 | 1.50 |
| | | | | | GFR, arbitrary unit | 100 | 140 | 1.40 |
| 8 | Brattleboro DI rats: Control vs. dDAVP injections | 386 ± 12 | 431 ± 31 | 1.20 | | | | |
| 11 | Brattleboro DI rats: Control vs. dDAVP infusion | 555 ± 11 | 758 ± 42 | 1.37 | | | | |
| 10 | Brattleboro DI rats: Control vs. dDAVP infusion | 338 ± 8 | 404 ± 13 | 1.20 | | | | |
| 2 | Wistar rats: High water intake vs. dDAVP infusion | 457 ± 15 | 681 ± 16 | 1.49 | | | | |
| 12 | Spr. Dawley rats: high water intake vs. dDAVP infusion | — | — | — | $^{14}C$ $C_{In}$, ml/min in 24 hr periods | 1.08 ± 0.07 | 1.91 ± 0.09 | 1.77 |
| 13 | Mongolian Gerbils with or without drinking water | 359 | 533 | 1.49 | | | | |
| 14 | Mongrel dogs: control vs. 36 hr water deprivation (with normal food intake) | | | | RBF, ml/min | 81.3 ± 3.1 | 89.0 ± 3.7 | 1.10 |

| Ref. | Species and conditions: Low vs. normal or high protein intake | Kidney weight *mg/100 g BW*: Low | High | High/low | Renal hemodynamics: Unit | Low | High | High/low |
|---|---|---|---|---|---|---|---|---|
| Alterations in protein intake | | | | | | | | |
| 53 | Normal rats: 18 vs. 55% casein | 379 | 473 | 1.25 | | | | |
| 54 | Normal rats: 18 vs. 67% casein | 357 | 548 | 1.54 | | | | |
| 48 | Dogs: low vs. high protein | — | — | — | GFR after a meal | 74.8 | 135.0 | 1.80 |
| 48 | Dogs: low vs. high protein | | | | GFR, post absorptive period | 37.1 | 83.8 | 2.26 |
| 42 | Munich Wistar rats: 6 vs. 40% casein | 374 | 456 | 1.22 | $C_{In}$, ml/min × 100 g BW | 0.321 | 0.435 | 1.36 |
| 55 | Sprague-Dawley rats: 6 vs. 50% protein | 308 ± 6 | 407 ± 5 | 1.32 | | | | |
| 41 | Sprague-Dawley rats: 10 vs. 32% casein | 424 ± 13 | 497 ± 15 | 1.17 | $C_{Cr}$, ml/day × 100 g BW | 586 ± 19 | 730 ± 12 | 1.25 |
| 40 | Sprague-Dawley rats: 10 vs. 32% casein | 658 ± 15 | 760 ± 25 | 1.16 | | | | |
| 49 | Wistar Furth rats: 6 vs. 23% casein | — | — | — | $C_{In}$, ml/min | 0.76 ± 0.01 | 1.82 ± 0.17 | 2.39 |
| 49 | Wistar Furth rats: 6 vs. 23% casein | | | | $C_{In}$, ml/min × 100 g BW | 0.54 | 0.85 | 1.57 |
| 50 | Bedouin goats: Wheat stray vs. Lucerne hay (0.58% vs. 2.6% N) | — | — | — | $C_{Cr}$, ml/day × kg BW | 2260 ± 140 | 4850 ± 300 | 2.15 |
| 51 | Ewes: 4.9% vs. 14% protein | — | — | — | $C_{In}$, ml/min × kg BW | 1.26 ± 0.30 | 2.04 ± 0.38 | 1.62 |
| 52 | Young healthy humans: 0.64 vs. 1.20 protein/kg/day | — | — | — | $C_{Cr}$, ml/min × 1.73 $m^2$ | 73.6 ± 23.6 | 116.4 ± 19.9 | 1.58 |

Abbreviations are: DI, diabetes insipidus; GFR, glomerular filtration rate; RBF, renal blood flow; SNGFR, single nephron GFR; KW, kidney weight; BW, body weight; $C_{In}$, inulin clearance; $C_{Cr}$, creatinine clearance.

unnoticed, probably because most experimental protocols designed to evaluate the influence of vasopressin on renal hemodynamics explored situations which were too extreme (infusion of vasopressin after prior induction of water diuresis, or increase in endogenous vasopressin by severe dehydration) or for too short periods of time to reveal significant effects [15]. Water diuresis or prolonged dehydration leads to severe perturbations of fluid balance which bring into play another set of influences on renal hemodynamics. We observed that ADH-dependent increases in GFR can be revealed only within a relatively narrow range of physiologic regulation, when changes in plasma and extracellular fluid volumes are prevented or are only moderate, or in chronic situations such as prolonged reduction (not suppression) of water intake, which enables a new steady state to be reached [15].

*Relative development of the different renal zones and nephron segments.* Concentration-induced hypertrophy of the kidney is not uniform. The inner stripe of the outer medulla (IS) is far more hypertrophied than the other zones of the kidney. As a result, the IS occupies a greater fraction of the total length of the cortico-medullary axis (Fig. 1), and the nephron segments and vessels located specifically in this zone become relatively longer than other structures in the hypertrophied kidney. This provides a

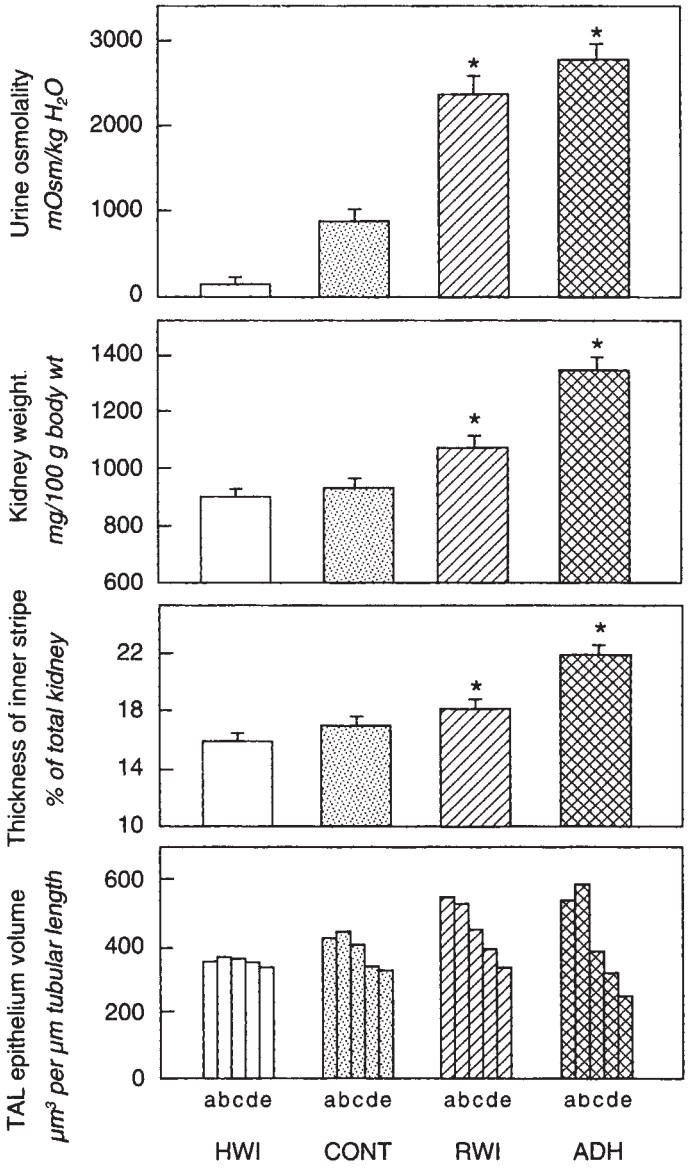


**Fig. 1.** *Influence of urinary concentrating activity on kidney size and morphology.* Chronic study in Wistar rats. Abbreviations are: HWI, rats with increased water intake; CONT, control rats; RWI, rats with water restriction; ADH, rats receiving a constant infusion of dDAVP. The volume of TAL epithelium per unit tubular length (bottom part) was measured at different levels along the TAL, including deep (a), mid (b), and superficial (c) parts of the inner stripe, mid outer stripe (d), and mid cortex (e). *Significantly different from CONT by ANOVA. Adapted from [2].

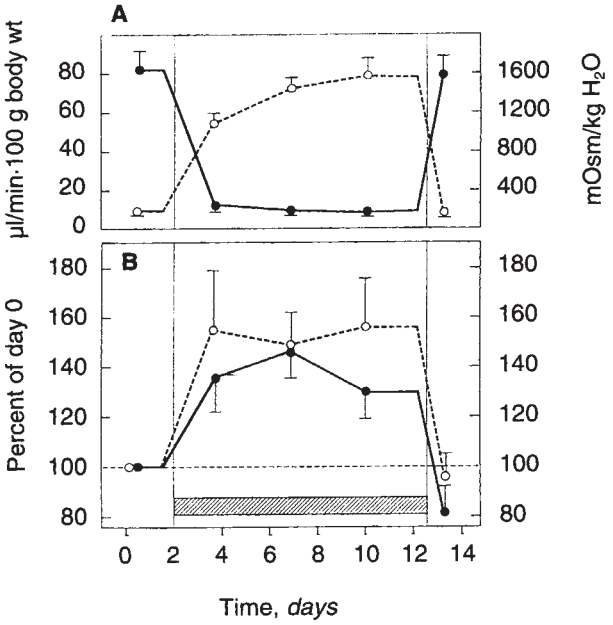


**Fig. 2.** *Influence of a chronic ADH infusion on urinary concentrating activity* (**A**) *and renal hemodynamics* (**B**) *in Brattleboro rats with diabetes insipidus.* Note the reversibility of the effects upon cessation of the infusion. In A symbols are: (○----○) urinary osmolality; (●——●) urinary flow rate; in B: (○----○) plasma flow rate, (●——●) GFR; (▨) vasopressin infusion. Adapted from [9]; used with permission.

greater length for countercurrent multiplication between thick ascending and thin descending limbs of Henle's loops and for countercurrent exchanges between ascending and descending structures in the vascular bundles, thus resulting in a more efficient machinery for urinary concentration and in better medullary insulation [16].

In addition to this lengthening, the sustained stimulation of urine concentrating activity modifies the proportion of thick ascending limb (TAL) tissue present in the different renal zones. TAL of well hydrated or control rats exhibit a relatively uniform volume of epithelium from the depth (IS) to the surface (cortex). In contrast, that of rats with increased sollicitation of urinary concentrating processes is markedly enlarged in the IS and thinned in the cortex (Fig. 1) [2]. Taking into account both the elongation of the IS and the thickening of the medullary TAL (MTAL), total TAL tissue per rat increased markedly with increasing urinary concentration, from 12.9 to 21.7 $mm^3$/100 g body wt, and the fraction of TAL epithelium located in the IS grew from 37 to 65% of total TAL epithelium volume per nephron (in rats with increased water intake and rats receiving ADH infusion, respectively) [16]. Most of the MTAL hypertrophy results from enlargement of pre-existing cells and not from cell proliferation. In the enlarged cells, mitochondrial volume and the area of the basolateral membrane increase homogeneously in proportion to cell volume. The incidence of binucleated cells (a sign of intense metabolic activity) in the wall of the MTAL is markedly increased [11].

The MTAL hypertrophy is accompanied by parallel increases in hormone-stimulated adenylate cyclase activity, Na-K-ATPase activity [8], and electrolyte transport capacity [17] (Table 2). The changes in enzyme activities per unit length of tubule in the medullary TAL are proportional to the hypertrophy, suggesting that they result from an increase in basolateral membrane surface area rather than from an increase in the density of enzyme molecules in this membrane (Table 2). The parts of the TAL lying in the outer stripe and in the cortex do not exhibit changes in volume or enzyme activities; if anything, these factors tend to be reduced (Fig. 1) [2, 8, 11, 18].

The "diluting segment" of the amphibian kidney (equivalent to the mammalian TAL [19]) adapts to changes in ambiant osmolality very similarly to the MTAL in rats. Salamanders conditioned

**Table 2.** Influence of alterations in water intake and/or ADH level, or in protein intake, on enzyme activity and transport in specific nephron segments

| Ref. | Species and conditions | Parameter | | Ratios high/low in nephron segments: MTAL | CTAL | CCD | MCD |
|---|---|---|---|---|---|---|---|
| Alterations in urinary concentrating activity | | | | | | | |
| 8 | Brattleboro DI rats: dDAVP-treated vs. controls | ADH-dependent ADC | per unit length | 1.73[a] | 0.55[a] | 0.83 | 0.95 |
| | | Glucagon-dependent ADC | " " " | 1.76[a] | 0.74[a] | 0.44[a] | 0.22[a] |
| | | Na-K-ATPase activity | " " " | 1.69[c] | 1.26 | 2.17[a] | 1.19 |
| | | · *Volume of epithelium* | " " " | 1.66[a] | | | |
| | | ADH-dependent ADC | per unit volume | 1.02 | | | |
| | | Glucagon-dependent ADC | " " " | 1.13 | | | |
| | | Na-K-ATPase activity | " " " | 1.11 | | | |
| 17 | Brattleboro DI rats: AVP-treated vs. controls | Transepith. potent. diff. | per unit length | 2.40[c] | | | |
| | | Chloride flux | " " " | 2.10[a] | | | |
| | | · *Volume of epithelium* | " " " | 1.15[a] | 0.99 | 1.01 | 1.00 |
| 18 | Gerboa, dehydrated vs. normally hydrated | Na-K-ATPase activity | per unit length | 1.30[c] | 1.01 | 1.46[c] | 1.06 |
| | | · *Volume of epithelium* | " " " | 1.27[c] | | | |
| | | Na-K-ATPase activity | per unit volume | 0.98 | 1.02 | 1.43 | 1.07 |
| 20 | Salamander living in pond water vs. distilled water | Transepith. potent. diff. | per unit length | 1.48[a] } | diluting segment | | |
| | | Na-K-ATPase activity | " " " | 1.82[a] } | | | |
| | | · *Volume of epithelium* | " " " | 1.28 } | | | |
| Alterations in protein intake | | | | MTAL Inner stripe | MTAL Outer stripe | Loop of Henle (= MTAL + CTAL) | |
| 40 | Sprague-Dawley rats: 32% vs. 10% casein | Tubule radius | | 1.37[b] | 1.10 | | |
| | | Na-K-ATPase activity | per unit length | 1.49[b] | 0.96 | | |
| 43 | Sprague-Dawley rats: 40% vs. 6% casein | NA reabs. in loop of H. (micropuncture) | | | | 1.22[b] | |
| | | Na-K-ATPase activity | per unit length | 1.87[b] | | | |
| 57 | Sprague-Dawley rats: 40% vs. 6% casein | Na reabs. in loop of H. (micropuncture) | | | | 1.40[b] | |

For each parameter, the table shows the ratio of values found in "High" vs. "Low" condition.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$
Abbreviations are: ADC, adenylate cyclase activity; H, Henle; MTAL and CTAL, medullary and cortical thick ascending limb, respectively; CCD and MCD, cortical and medullary collecting duct, respectively.

to live in distilled water instead of pond water exhibit a larger diluting segment, together with increased Na-K-ATPase activity, transepithelial potential difference, and ouabain binding [20] (Table 2). This adaptation helps conserve solutes by enhancing the capacity of the kidney to dilute urine. In mammals, the hypertrophy and enhanced activity of the TAL also increase the urinary diluting power. This is illustrated by the fact that the diabetic condition of Brattleboro rats becomes worse after cessation of chronic treatment with ADH than that of never treated DI rats (urine osmolality ≈80 mOsm/kg $H_2O$ vs ≈170 in never treated DI rats, and urinary flow rate ≈125 ml/24 hr × 100 g body wt. vs. ≈65) [21]. This changes were seen to last for several weeks (M.M. Trinh-Trang-Tan, N. Bouby and L. Bankir, personal observation).

The impaired solute reabsorption by the thick ascending limb in ADH-deficient rats lead to an increased delivery of NaCl to the next nephron segment, the distal convoluted tubule (DCT) [22, 23]. As a consequence of this increased load, Na and Cl transport in this segment is permanently enhanced, resulting in thickening of the epithelium, increase in the number of cells per cross section, and elongation [24]. Chronic treatment with dDAVP, increasing transport in the loop of Henle and thus decreasing the load to the DCT, was followed by a decrease in epithelium height and number of cells per cross section, to values close to those seen in normal rats [24].

*Internephron heterogeneity.* The above-mentioned urinary concentration-dependent increases in kidney weight and in GFR are unequally distributed among nephrons. In most mammals, juxtamedullary nephrons have a larger glomerulus and a longer proximal tubule, and exhibit a higher single nephron filtration rate (SNGFR) than superficial nephrons [25]. This normal heterogeneity is absent in Brattleboro rats and can be restored to almost normal by chronic ADH treatment [6, 7]. Alterations in urinary concentrating activity were also shown to influence nephron heterogeneity in normal rats [2]. Additional experiments in mice with nephrogenic DI showed that the usual nephron heterogeneity does not depend on vasopressin *per se,* but rather on the

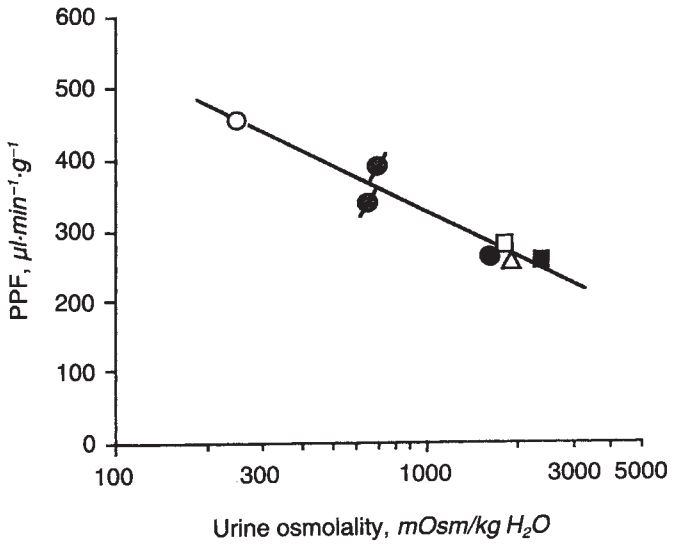


**Fig. 3.** *Influence of urinary concentrating activity on papillary plasma flow rate (PPF) measured by the albumin accumulation technique (note the log scale for the abcissa).* Symbols are: (○) DI, homozygous Brattleboro rats with diabetes insipidus; (△) HZ, heterozygous Brattleboro rats (controls for the DI); (◐) DI rats receiving AVP or dDAVP acutely (30 min); (□) control Wistar rats; (■) Wistar rats dehydrated for 2 days; (●) DI rats receiving dDAVP chronically (5 days). Each point is the mean of 6 to 15 rats. A group of Wistar rats was dehydrated. Adapted from data in [10]; used with permission.

efficient operation of the urinary concentrating processes. These mice also exhibit significantly reduced nephron heterogeneity, although they have normal (or even increased) circulating vasopressin levels [26].

*Other urinary concentration-induced changes.* The hormonal regulation of medullary hemodynamics is still incompletely understood. The renal medulla includes several different vascular compartments and most available techniques cannot investigate them all adequately. Most studies dealing with the influence of ADH on "medullary" blood flow actually concern only the tip of the papilla, that is, the most extreme part of the medulla, and provide no information on the influence of this hormone on blood flow to the outer medulla, where the active, energy-demanding steps of the urinary concentrating process take place [27].

Homozygous Brattleboro rats with DI exhibit a high papillary plasma flow rate (PPF) compared to heterozygous rats of the same strain or to normal Wistar rats ($461 \pm 26$ vs. $270 \pm 16$ $\mu l \cdot min^{-1} \cdot g^{-1}$, $P < 0.001$). After chronic dDAVP administration, PPF decreases in proportion to the concomitant increase in urine osmolality (Fig. 3) [10]. This influence does not seem to depend on the vascular effects of vasopressin since a non-pressor agonist is able to induce these changes [10].

The availability of ADH has been shown to exert a marked influence on the urinary excretion of divalent cations, calcium and magnesium [21]. DI rats exhibit an abnormally high excretion of these cations which is dramatically lowered by chronic ADH infusion, without altering that of sodium or potassium [21]. Micropuncture and microperfusion experiments have localized these effects to the cortical thick ascending limb [22, 28].

Finally, the prolonged absence or presence of ADH has been shown to influence (indirectly in some cases) other aspects of renal function including prostaglandin synthesis [29, 30; reviewed in 31, 32], renin-angiotensin system [33, 34], composition of medullary interstitial tissue [35], and accumulation of osmolytes in renal cells [reviewed in 36].

### *Adaptation to protein intake: Similarities with the adaptation to urinary concentration*

That high protein (HP) intake increases renal hemodynamics and hypertrophies the kidney has been known for decades. The mechanisms responsible for these effects are, however, still not well understood in spite of intense research stimulated by the desire to understand why and how the level of protein intake influences the progression of chronic renal diseases. Obviously, several factors are involved simultaneously and/or successively. The aim of this review is to analyze specifically the possible contribution of factors related to protein-induced changes in urea excretion and in water balance. Because of the role played by urea in the process of water conservation, we think that these factors should bring essential clues to our understanding of the mechanisms by which the kidney adapts to changes in the level of protein intake. As will be described below, several findings point to a major role of ADH and urinary concentrating processes in inducing the protein-induced changes in renal function and anatomy. A number of other biochemical, humoral, and nervous factors which are certainly also involved in the renal adaptation to protein intake will not be addressed here. They have recently been reviewed by other authors [37–39].

A striking similarity is observed between protein- and concentration-induced changes in renal function and morphology, as illustrated in Figure 4. This figure displays the results of several independent studies performed either by varying ADH and/or water availability on the one hand [2, 8, 12, 17], or the level of protein intake on the other [40–43].

The influence of the level of protein intake on kidney weight and renal hemodynamics is well documented (Table 1) [41–52]. Typically, kidney weight relative to body weight is about 20 to 50% greater in animals fed a protein-rich versus a protein-poor diet [40–42, 53–55] and the kidney enlargement is roughly proportional to the protein content of the diet [44]. The effect of HP intake is cumulative with that of many other stimuli for renal growth [56].

Most interesting is the observation that the protein-induced hypertrophy exhibits the same intrarenal pattern as that induced by ADH and stimulation of urinary concentrating activity (Fig. 4). The IS is lengthened along the cortico-medullary axis by 54% versus only 12% for the kidney as a whole [41]. This preferential increase of the IS seems specific to conditions when the stimulus for kidney enlargement is linked to protein intake and/or urinary concentration, since in other situations involving an increase in kidney weight such as normal growth with age or compensatory hypertrophy, all kidney zones enlarge in fairly equal proportions [41].

Within the IS, the MTAL hypertrophies about 50% more than expected from the overall increase in kidney mass. This hypertrophy is more pronounced in the earliest part of this segment and is not observed in the outer stripe or in the cortex [41], a situation very similar to that described after chronic stimulation of urinary concentration. *In vivo* micropuncture and loop microperfusion experiments have provided strong evidence that the transport activity of the TAL is markedly increased after high protein intake

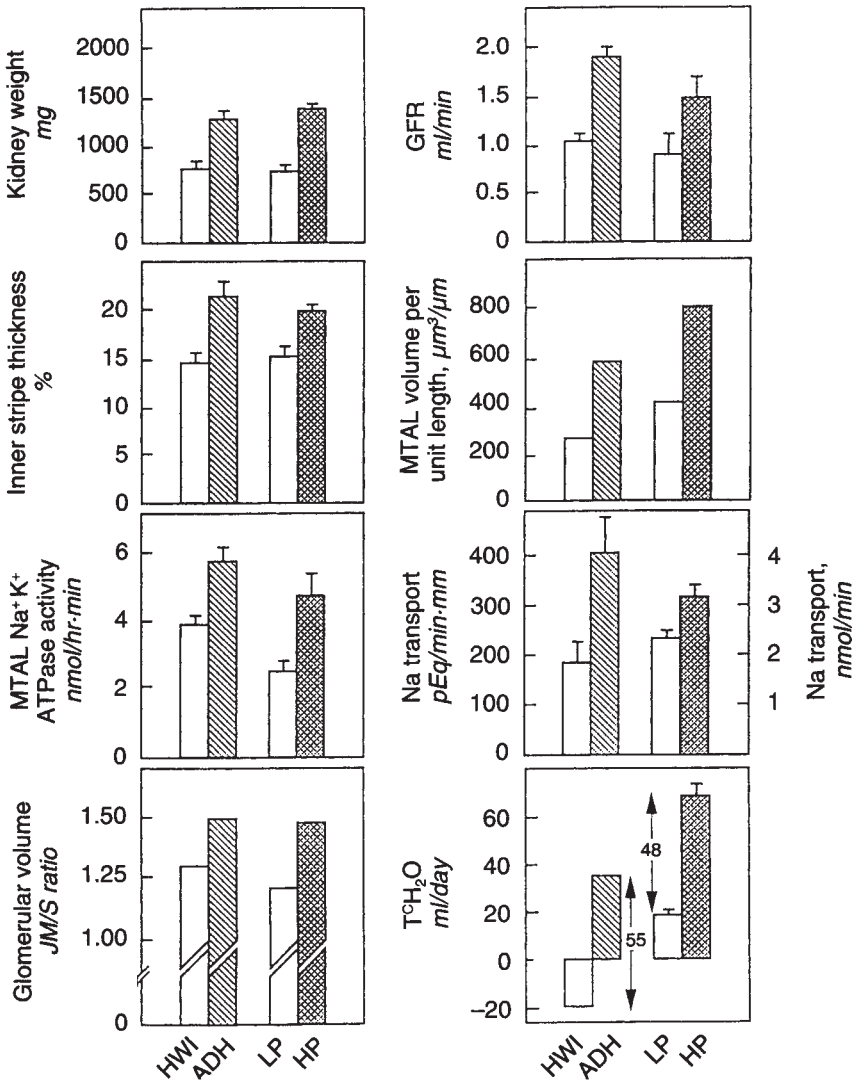


**Fig. 4.** *Similarity of the effects induced in kidney morphology and function by chronic alterations in either urinary concentrating activity (ADH vs. HWI) or protein intake (HP vs. LP).* Abbreviations are: HWI, Brattleboro rats with DI or Wistar rats with increased water intake (that is, low urinary concentrating activity); ADH, Brattleboro or Wistar rats receiving a constant infusion of dDAVP; LP, low protein diet (6 or 10% casein); HP, high protein diet (32 or 40% casein). Adapted from [2, 8, 12, 17] for HWI/ADH, and [40–43] for LP/HP.

[57], a change that is paralleled by an increase in Na-K-ATPase activity per unit tubular length in the MTAL [40, 43] (Fig. 5 and Table 2).

The parallelism between protein- and concentration-induced changes also concerns internephron heterogeneity. High protein feeding accentuates the difference in glomerular volume between superficial and juxtamedullary nephrons [41]. It may be reasonably assumed that the difference between superficial and deep nephron SNGFR is also enhanced, although such measurements are not available. The finding by Seney and Wright of a higher increase in whole kidney GFR than in superficial nephron GFR (29 vs. 21%, respectively) is compatible with this view [58].

## Urea excretion and water conservation: Crucial factors in the vasopressin- and protein-induced changes in renal function

### *Vasopressin and urea*

No active transport of urea seems to be involved in the urinary concentrating mechanism [59]. Nonetheless, urea is concentrated to a very high extent in urine with respect to plasma and plays a crucial role in the urinary concentrating mechanism. By its accumulation in the inner medulla (IM), urea contributes to enhance the osmotic pressure gradient able to subtract water from the collecting ducts (CD) [59], thus explaining why urea has the unique property of inducing "an economy of water in renal function" as stated by Gamble et al sixty years ago [60]. Note that this economy occurs only within certain limits of the urea/non-urea solute ratio in the urine [61].

Urea accumulation in the IM is critically dependent on the V2 effects of ADH on the CD in two ways. (1) By increasing water permeability in the cortical and outer medullary CD, ADH favors urea concentration in the lumen of the CD. (2) In the terminal part of the IMCD, ADH enhances the permeability of the epithelium to urea, thus enabling urea (concentrated upstream) to diffuse into the papillary interstitium [59, 62]. In the absence of ADH (after induction of intense water diuresis or in Brattleboro rats with DI), the accumulation of sodium in the IM is close to normal, wheras that of urea is totally abolished [63, 64]. Upon reintroduction of ADH, urea accumulation in the IM is a relatively slow process, and several hours (after water diuresis) or days

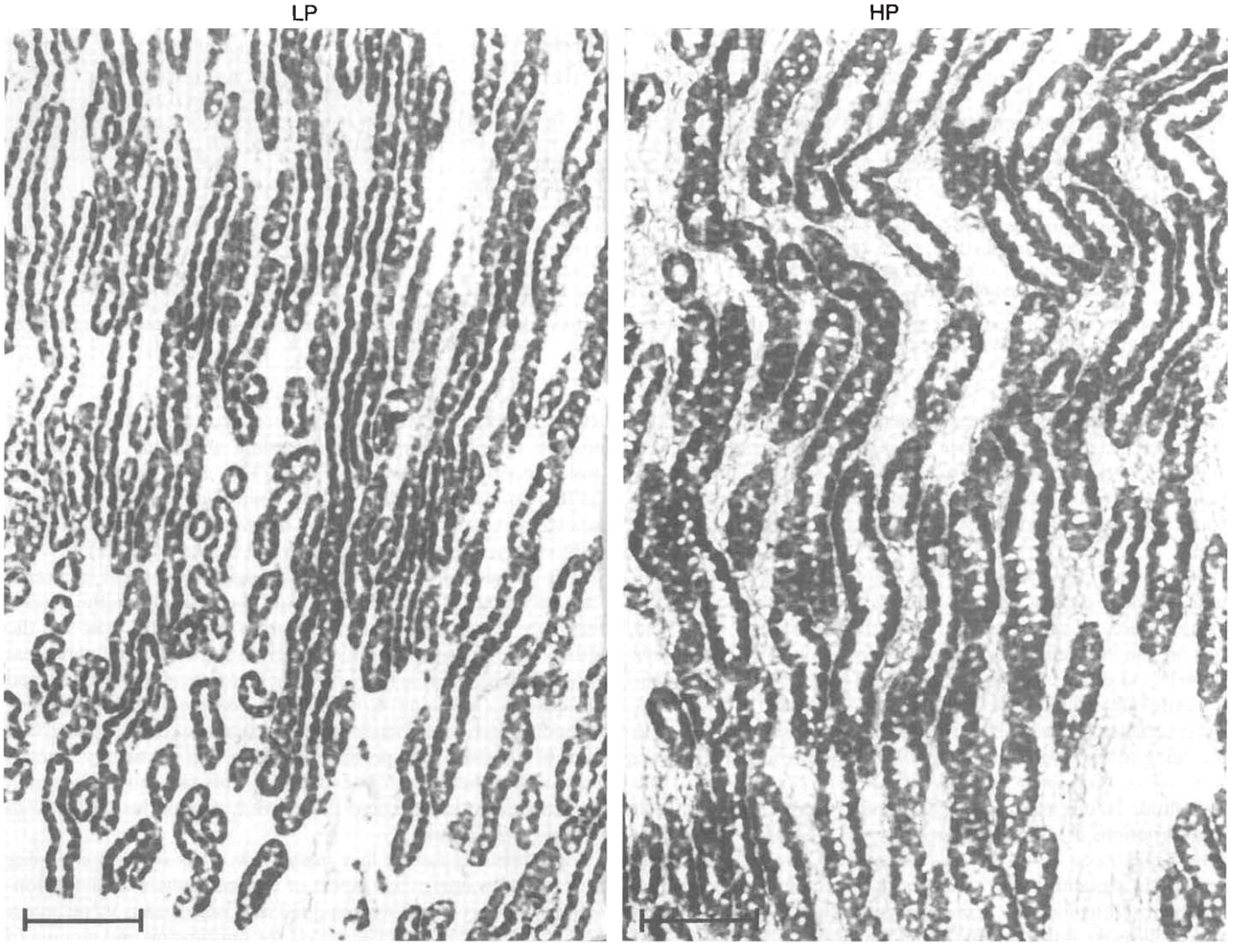


**Fig. 5.** *Influence of protein intake on Na-K-ATPase activity and epithelium thickness in Sprague-Dawley rats fed 10 or 32% casein diets (LP and HP, respectively).* Longitudinal sections of kidneys showing the inner stripe of the outer medulla after a histochemical reaction revealing ATPase activity. The density of the enzyme activity per unit cell surface area in the thick ascending limbs was unchanged but the size of the cells, the epithelium volume and hence the Na-K-ATPase activity per unit length of tubule (as well as sodium chloride reabsorption, see text) were markedly increased. Bars = 100 $\mu$m. Reproduced from [40].

(after diabetes insipidus) are required to reach maximal urinary osmolality [64–66].

Actually, urea tends to be continuously washed out from the IM by the blood flowing in the venous, ascending vasa recta. Countercurrent exchanges between ascending and descending vasa recta limit this wash-out. In addition, a complex recycling route brings back part of this urea to the IM as follows. In the outer medulla, urea diffuses by countercurrent exchange from ascending vasa recta into the descending branch of the nephrons (thin descending limbs of short looped nephrons and possibly pars recta of all nephrons). Urea is then concentrated in the lumen of the distal nephron by water abstraction and returns to the IM by diffusion from the terminal CD. This recycling process renders urea accumulation in the IM more efficient and thus improves urinary concentrating ability. However, it has two major consequences which have not been given much attention in the last decades, but might be of great importance in understanding the adaptation of the kidney to chronic high urinary concentration.

*Consequences of the vasopressin-dependent intrarenal urea recycling.* The first consequence is that vasopressin-dependent urea recycling markedly reduces the efficiency of urea excretion since it subtracts from the urine some of the urea flowing in the terminal CD and reintroduces it into the nephron. Moreover, vasopressin-dependent water reabsorption in cortical and medullary CD slows the urine flow rate and increases the transepithelial gradient of urea concentration, two factors which increase passive urea reabsorption along the whole CD [67, 68]. These combined effects markedly reduce the fractional excretion (FE) of urea. At high urinary flow rates, about 60% of the filtered urea is excreted in the urine whereas at low urinary flow rates, $FE_{urea}$ falls to only $\approx$20% [68]. As a result, three times more urea needs to be filtered at low than at high urinary flow rates in order to ensure the same urea

**Table 3.** Influence of papillectomy on kidney weight (KW) and glomerular filtration rate (GFR)

| Ref. | Rat strain and protocol | Parameter | Control | Papillect. | Pap/Cont |
|---|---|---|---|---|---|
| 74 | Wistar rats. anesthetized, SPX, 2–8 weeks | GFR, ml/min (1 K) | 1.06 ± 0.22 | 0.58 ± 0.18 | 0.55 |
| | | KW, g | 1.50 ± 0.27 | 1.03 ± 0.26 | 0.69 |
| 75 | Wistar rats, anesthetized, SPX, 3–6 weeks | GFR, ml/min × kg BW (1 K) | 3.03 ± 0.24 | 1.69 ± 0.16 | 0.56 |
| | | KW, g | 1.23 ± 0.05 | 1.03 ± 0.04 | 0.83 |
| 73 | Wistar rats, anesthetized, CPX, 5–7 weeks | GFR, ml/min × 100 g BW | 1.25 ± 0.09 | 0.80 ± 0.13 | 0.64 |
| 72 | SHR rats, anesthetized, CPX, 12 weeks | GFR, μl/min × 100 g BW | 701 ± 48 | 459 ± 30 | 0.65 |
| | | KW, per unit BW | | | 0.90 |
| 71 | Wistar rats, anesthetized, CPX, 6–10 days | GFR, ml/min (sum of 2K) | 2.1 ± 0.1 | 1.4 ± 0.1 | 0.67 |
| 76 | Wistar rats, anesthetized, CPX, 24 hrs | GFR, ml/min (sum of 2 K) | 1.97 ± 0.14 | 1.16 ± 0.14 | 0.59 |
| 77 | Sprague-Dawley rats conscious, CPX, 2 days | GFR, ml/min (sum of 2 K) | 2.52 ± 0.14 | 2.13 ± 0.09 | 0.84 |
| 70 | Wistar rats conscious, CPX, 24 hrs | GFR, μl/min × 100 g BW | 895 ± 69 | 584 ± 26 | 0.65 |

Abbreviations are SPX, unilateral surgical papillectomy vs. contralateral normal kidney; CPX, chemical papillectomy with bromoethyleneamine vs. control group; K, kidney; BW, body weight.

excretion. In this context, the increase in renal hemodynamics seen after sustained stimulation of urinary concentrating activity (reviewed above) contributes to increasing the amount of urea filtered. As a result, plasma urea concentration increases less than would be expected from the ADH-dependent reduction in $FE_{urea}$ [69].

The second consequence is that the variable intensity of the urea recycling process probably results in wide variations in the concentration of urea flowing in the thick ascending limb, and thus in the transepithelial urea concentration gradient in this segment. As deduced from micropuncture studies of the accessible early distal tubule, the tubular fluid-to-plasma urea concentration gradient in the late TAL probably varies between 3 and 10, according to the level of urea recycling. With high urea recycling, urea may account for as much as half of the solutes present in late TAL fluid. To our knowledge, the possible consequences of such large variations in luminal concentration of urea on TAL function have never been investigated. We recently proposed a novel hypothesis explaining how vasopressin-dependent urea recycling and the resulting increase in urea concentration in the TAL lumen could indirectly influence the tubuloglomerular feedback signal (TGF) and thus alter GFR [69]. In brief, urea, acting as an osmotic buffer in the lumen, could reduce an osmotically driven water leakage from the TAL and thus could enable a lower NaCl concentration to be reached in the fluid passing by the macula densa.

In this hypothetical mechanism, vasopressin-dependent urea movements within the kidney would be responsible for an increase in renal hemodynamics. This original concept is supported by the following observation. A number of independent studies all show that papillectomy (surgical removal or chemical destruction of inner medullary tissue), which reduces urinary concentrating capacity and increases urinary flow rate, is followed by a dramatic fall in both GFR and kidney weight (Table 3) [70–77]. These reductions do not seem to be a mere consequence of diminished nephron number since no more than 10 to 15% of all nephrons have a long loop reaching the papilla and are thus likely to degenerate after papillectomy. Moreover, hyperfiltration and hypertrophy of non-damaged superficial nephrons could easily bring renal function and weight back to normal, as observed after partial nephrectomy. Papillectomy obviously compromises urea recycling by suppressing or damaging the terminal CD where the initial step of this recycling occurs. It may be assumed that the absence of urea recycling (1) permits an efficient urea excretion (high fractional excretion), and (2) reduces the concentration of urea in the TAL lumen thus possibly abolishing the putative urea-dependent influence on TGF. This could explain the low GFR, reminiscent of that seen in Brattleboro rats or in normal rats where urinary concentrating activity and urea recycling are reduced by a chronic increase in water intake (Table 1).

If it is confirmed that vasopressin and the resultant intrarenal urea recycling indeed induce a chronic hyperfiltration, the natural tendency to produce hypertonic urine has a high cost for the kidney. The price paid for this efficient water conservation process is not limited to the energy spent in concentrating the daily load of osmoles (involving active sodium reabsorption in the thick ascending limb) but also includes consequences on the glomerular filter of sustained high pressure and flow, and the energy spent in reabsorbing the extra solutes filtered. This favorable adaptation in terms of evolution could thus have a deleterious influence in chronic renal failure.

*Vasopressin action on liver metabolism.* It is worth mentioning here another, intriguing, aspect of the vasopressin-urea relationships. Hepatocytes are well equiped with vasopressin $V_1$ receptors [78] (the liver has even been used for purification and cloning of this receptor [79]). *In vitro,* hepatocytes respond to vasopressin by an increased metabolic activity including enhanced gluconeogenesis and ureagenesis, effects that are additive to those of glucagon [80–83]. The physiologic relevance of vasopressin action on liver metabolism has not yet been established *in vivo.* However, if these effects occur for physiologic levels of the hormone, it is tempting to assume that vasopressin, in addition to enhancing intrarenal urea recycling, might actually also increase the amount of urea delivered by the liver to the kidney.

### *Protein intake, urea, and vasopressin*

An increase in protein intake above the minimum requirement for maintaining nitrogen balance will induce a roughly parallel increase in the excretion of urea, the major end-product of protein catabolism. Since urea is far more concentrated in urine than in plasma (≈20- to 50-fold in humans, several hundred-fold in rodents), the "osmotic work" that the kidney must accomplish in order to excrete urea in a reduced amount of water increases in proportion to the increase in protein intake. Figure 4 shows that free water reabsorption rises with protein intake as it does after ADH administration [84]. The striking similarity of the changes induced in kidney function and morphology either by a high protein diet or by ADH infusion strengthens the notion that ADH

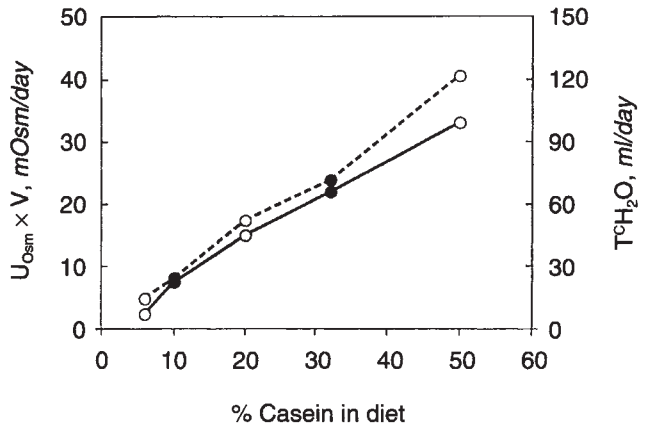


**Fig. 6.** *Influence of protein intake on solute excretion (dotted line) and free water reabsorption (solid line).* Data from two studies in Sprague Dawley rats are combined (○, [41]; ●, [91]). Urea accounts for the largest part of the increase in solute excretion with increasing protein intake. Free water reabsorption (that is, the amount of water that the kidney reabsorbs to concentrate urinary solutes at the observed osmolality) is also increased in parallel. Reproduced from [84]; used with permission.

and/or the consequences of its action on the kidney play a decisive role in the renal response to dietary proteins. Three relevant questions may be considered. How does the level of protein intake and the ensuing changes in urea excretion influence water metabolism and the kidney's "osmotic work" (as defined by Addis [85, 86])? How does the kidney of ADH-deficient Brattleboro rats react to high protein intake? Do changes in protein intake affect circulating ADH level?

*Relationships between protein intake, and urea and water excretions.* As recalled above, urea concentration is permitted by the recycling of urea and its accumulation in the renal medulla. The intensity of urea accumulation in the medulla, and hence of urea concentration in the urine, critically depends (1) on the level of ADH and (2) on the amount of urea available. Medullary urea accumulation is impaired when ADH is depressed, as in water diuresis [63], or absent, as in Brattleboro rats [64], and is restored by a few days of ADH infusion (this restoration requires a longer time when ADH is not infused continuously but is given as daily injections [7, 87]). On the other hand, medullary accumulation of urea is reduced when the amount of urea to be excreted is low. This explains why urinary concentrating ability is impaired by low protein intake and protein malnutrition [88]. Conversely, high protein intake, increasing urea delivery to the kidney, increases maximum urinary concentrating ability [41, 89, 90]. Note that even on *ad libitum* fluid intake (that is, when no attempt is made to induce maximum concentrating capacity), the kidney's spontaneous concentrating activity is also significantly enhanced by a high protein diet, as illustrated in Figure 6 [41, 91].

We observed that high protein diet increases osmolar excretion and the reabsorption of free water ($T^cH_2O$) in a very parallel fashion (Fig. 6). This means that the kidney reabsorbs more water during high than during low protein intake, in order to concentrate a larger amount of urea. Maintaining the medullary osmotic pressure gradient in the face of an increased solute excretion obviously requires a more intense transport activity in the nephron segment involved in the separation of salt from water, that is, the TAL. Therefore, the increase in $T^cH_2O$ seen on a high protein intake is likely the result of an equivalent increase in reabsorptive work of the TAL. Knowing this relationship, the enhanced sodium chloride reabsorption measured in the loop of Henle [57], and the selectively greater hypertrophy [41] and enhanced Na-K-ATPase activity [40, 43] observed in the TAL on a high protein intake (Fig. 5) are obviously related to the increased power necessary for enhancing free water reabsorption in the face of an increased load of urea to be excreted in a concentrated urine. The mechanisms by which ADH could directly of indirectly influence TAL function will be discussed later in this review.

Besides the possible contribution of ADH, it is interesting to note that amino acids and glucagon, known to increase their concentration in the blood after a protein meal, could also contribute to enhancing both GFR and urinary concentrating activity by influencing TAL transport. First, certain amino acids have been shown to exert a vasodilatory effect on the kidney while improving urinary concentrating ability and preservation of TAL integrity during *in vitro* perfusion of isolated rat kidneys [92–96]. Second, in anesthesized dogs, i.v. infusion of the amino acid glycine was shown to increase GFR together with a marked increase in the metabolic rate of the outer medulla, likely ascribable to an increase in active transport in the TAL [97]. Third, glucagon has been shown to simultaneously enhance GFR and urinary concentrating ability even when ADH was maintained at a constant level [98, 99]. The latter effect most probably results from direct stimulation by this hormone of sodium chloride transport in the TAL [100]. In agreement with this possiblity, glucagon was shown to decrease the intensity of tubuloglomerular feedback [101] in a way similar to that observed after high protein intake [57].

*Influence of protein intake on the kidney in the absence of ADH.* Bouby and coworkers [102] showed that, in Brattleboro Di rats (that is, in the absence of ADH) the changes in renal function and anatomy usually induced by high protein intake are greatly reduced (modest increase in GFR) or even absent (no change in kidney mass or in the relative height of the inner stripe of the outer medulla; Fig. 7). This suggests that ADH (or the operation of the concentrating process) is necessary for full manisfestation of the effects of high protein intake on the kidney. On the other hand, Fernandez-Repollet et al [103] also studying the influence of protein intake on GFR and kidney weight in Brattleboro DI rats, concluded that these effects did not depend on ADH. However, the unequal growth of the rats in different groups fed different protein diets has not been taken into consideration in their study. Factoring GFR and kidney weight by body weight markedly reduces the observed differences [103], thus showing, as in Bouby et al's study that protein-induced changes are weakened in the absence of ADH.

Worthy of interest is the observation that, contrary to what occurs in normal rats with ADH, HP diet in Brattleboro DI rats induced no change in free water clearance or in the urine-to-plasma urea concentration ratio, in spite of the expected change in absolute urea excretion (Fig. 7). An acute injection of ADH restored a significant difference in the capacity to reabsorb free water between HP- and LP-fed DI rats, likely attributable to the greater availability of urea for ADH-induced urea accumulation and recycling in the medulla in HP-fed rats [102]. These observations underline the interdependence of urea and ADH in enabling the kidney to spare water.

The effects of proteins in normal rats are also blunted if high protein intake is associated with increased water intake intended

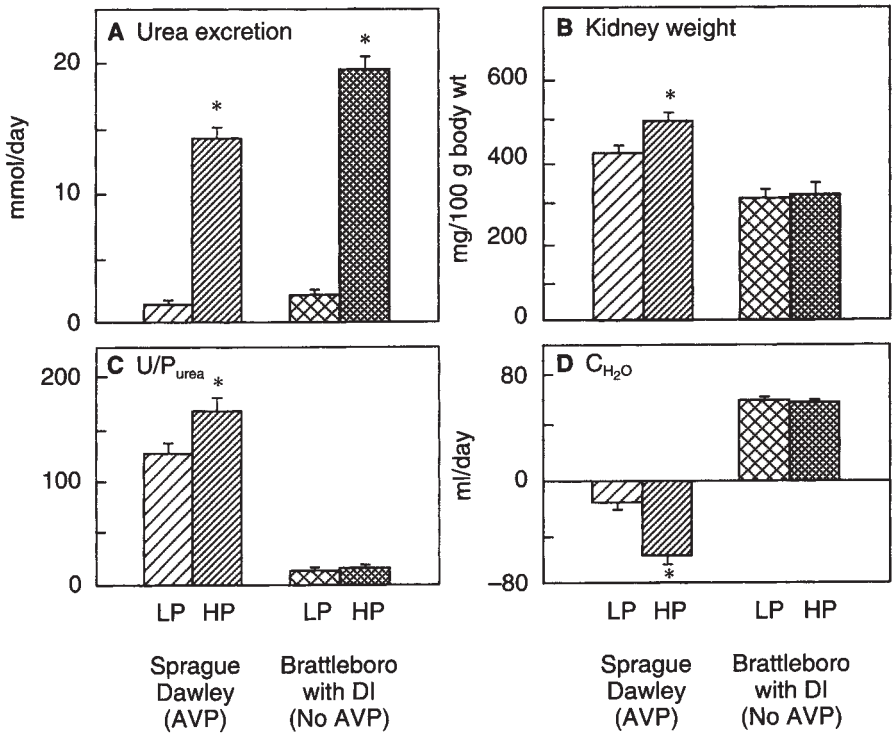


**Fig. 7.** *Influence of protein intake on the kidney in rats with (Sprague Dawley) or without antidiuretic hormone (homozygous Brattleboro rats with central diabetes insipidus).* Although high protein intake induced comparable enhancement of urea excretion **(A)** in the two strains of rats, kidney weight **(B)**, relative urea concentration in urine with respect to plasma (**C,** $U/P_{urea}$), and free water clearance (**D,** $C_{H_2O}$) did not change in the rats that were unable to concentrate their urine (*$P < 0.05$ or less vs. corresponding LP group) (adapted from [40, 41, 102]).

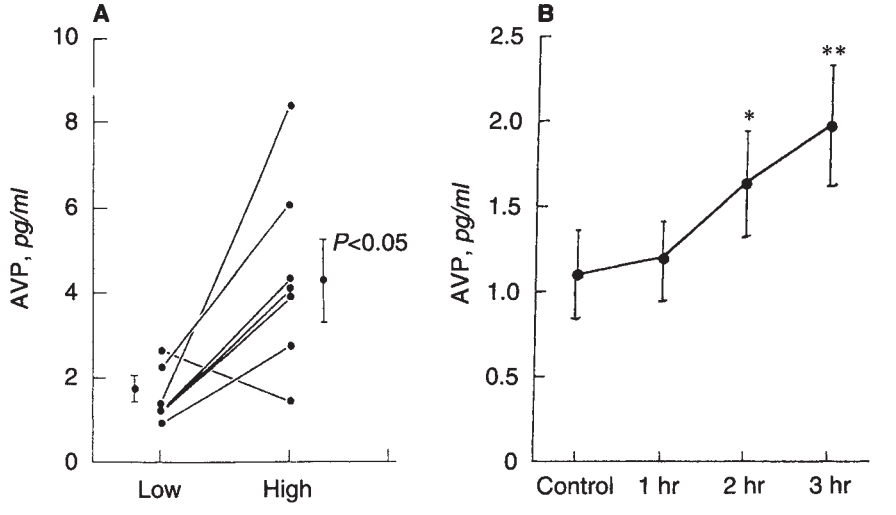


**Fig. 8.** *Influence of protein intake on plasma ADH concentration.* **A.** Chronic effects in healthy subjects on low or high protein intake for several days; reproduced from [106]. **B.** Acute effects in healthy subjects studied before (control) and during 3 hours after ingestion of a protein meal (* and ** = $P < 0.05$ and 0.01, respectively, vs. control period); adapted from [108].

to reduce urinary concentrating activity [104]. Conversely, they are intensified when ADH infusion is combined with high protein diet [51]. All these observations strongly suggest that ADH is involved—or at least plays a permissive role—in the protein-induced changes in GFR and kidney mass.

*Influence of protein intake on plasma ADH concentration.* Little information is available regarding the influence of protein intake on vasopressin plasma level. Changes in this hormone could, however, be suspected since the kidney's concentrating activity is obviously increased by a higher protein intake, as explained above. In 1964, using an ADH bioassay, Little and Radford reported that high protein intake in rats increased plasma antidiuretic activity in a chronic fashion, that is, even when measured at a distance from the last meal [105]. More recently, an investigation in normal volunteers showed that several days on a high protein intake increase plasma vasopressin 2.6-fold (Fig. 8) [106]. We also observed a higher AVP in rats fed 32 versus 10% casein diets (N. Bouby, L. Bankir and D. G. Bichet, unpublished results).

Two investigations in humans show that vasopressin plasma level increases about twofold in the two to three hours following a single protein meal (Fig. 8). Urine osmolality also increases simultaneously [107, 108]. The changes in plasma vasopressin and urine osmolality that occur in everyday life after ingestion of a protein meal are probably even greater than revealed by these

studies since they were carried out in a state of mild [108] or high [107] water diuresis achieved by frequent—non physiologic—fluid ingestion during pre- and post-meal hours.

The study of Hadj et al showed that the increase in plasma vasopressin seen after a meal strictly follows an increase in plasma osmolality (observed in spite of periodic hydration) [108]. Because plasma amino acid concentration may increase by several mmol/liter after the ingestion of a protein meal [109, 110], and because these amino acids probably exert an osmotic influence on sensitive cells responsible for vasopressin release, it may be assumed that an increase in the release of this hormone is a normal event during postprandial hours when the meal is rich in proteins. To our knowledge, the influence of alterations in plasma amino acid concentration on vasopressin release has not been studied.

## Possible common mechanism(s) responsible for adaptation of the kidney to urinary concentration and to protein intake

On the whole, the observations reviewed in the preceding sections have established (1) that the protein-dependent changes in GFR, kidney weight, and free water reabsorption are strikingly similar to those induced by chronic stimulation of urine concentration; (2) that ADH is required for the protein-dependent changes in renal function and morphology; and (3) that ADH plasma level is elevated by acute protein ingestion and by chronic high protein intake. These observations strongly suggest that protein- and concentration- (ADH) induced changes in kidney function and morphology share a common mechanism(s) and/or that ADH participates to a significant extent in the protein-induced effects.

These observations, however, do not reveal the common step that could initiate the changes induced by dietary proteins and/or ADH. Even if ADH secretion increases after protein ingestion, its contribution by a direct vasoactive effect on glomerular arterioles can be ruled out as the possible cause for the increase in GFR since (1) the change in GFR requires several hours and thus does not likely result from acute modulation of vascular tone [9], and (2) dDAVP, a nonpresssor analogue of AVP, induces this increase in renal hemodynamics [7, 111].

### *Increased transport in the TAL*

Because high protein intake increases urinary concentrating activity and induces a more prominent enlargement and increased activity of the nephron segment most directly involved in urinary concentrating activity, as does chronic stimulation of urinary concentration by vasopressin, it is tempting to imagine that an early event, common to both conditions, is stimulation of active transport in the TAL. The results of Seney and Marver show that the enhancement in loop NaCl reabsorption precedes the increase in medullary mass and could thus be the cause, rather than the consequence, of MTAL hypertrophy [43].

Actually, the TAL is ideally suited for exerting an effect on both GFR and urinary concentration since (1) it is located just prior to the macula densa and its fluid is thus susceptible to influencing the TGF control of GFR, and (2) it generates, by its active NaCl reabsorption, the "osmotic work" responsible for building up and maintenance of the osmotic pressure gradient in the medulla (Fig. 9). Whole kidney enlargement would follow the chronic increase in GFR in order to adapt the reabsorptive capacity of the kidney to the higher level of filtration [56] with, however, preferential hypertrophy of the renal zone (IS) and nephron segment (TAL) in which the initiating process took place. This scheme is compatible with experimental data of Seney, Persson and Wright showing that the mechanism responsible for the rise in GFR during high protein intake involves depression of the TGF due to a more intense reabsorption in the loop of Henle (in the TAL) and the subsequent lowering of NaCl concentration at the macula densa [57].

If stimulation of TAL transport seems a very likely common step in the protein- and concentration-induced changes in renal hemodynamics and size, the stimulus that initiates this increased transport remains unknown. A few possibilities will be discussed below.

### *Direct effect of ADH and/or glucagon on the TAL?*

Vasopressin and glucagon stimulate adenylate cyclase activity [112] and solute reabsorption in the TAL [22, 100, 113, 114]. Protein (or amino acid) ingestion stimulates glucagon [115, 116] and vasopressin (see above) secretion. It is thus logical to assume that, during chronic high protein feeding, these two hormones could contribute to enhancing TAL transport by their direct effect on this nephron segment. The significant increase in urinary osmolality and/or in free water reabsorption seen after infusion of physiological amounts of glucagon in rats favors this possibility for glucagon [98, 99, 117]. In contrast, a direct influence of vasopressin on TAL transport is probably not involved since the plasma level of this hormone required for stimulating this transport in the rat is relatively high [118] and since the functional consequences of vasopressin influence on TAL are detectable *in vivo* only after two days of water deprivation [119]. Nonetheless, because vasopressin increases concommittantly with glucagon after a protein meal, additive effects of the two hormones on TAL transport cannot be ruled out.

Apart from a possible direct hormonal influence, the morphological and functional changes of the TAL seem to result from a more complex sequence of events involving alterations in the composition of the tubular fluid and/or of the peritubular environment. The following examples demonstrate the influence of physico-chemical factors (independent of changes in hormonal concentrations) on epithelium transport and development. (1) Impressive structural and functional adaptation occurs in the salamander diluting segment when the solute conservation capacity of animals is challenged by immerging them in distilled water [20]. (2) A significant fall in enzymatic activity and cell mass is observed in a blinded intestinal loop whereas compensatory increases are seen in the next gut segment in which food transit was maintained and which had become more proximal [120]. Since the blood supply and thus the same hormonal environment were maintained in both loop segments, their opposite adaptations obviously resulted from changes in luminal delivery and not from possible alterations in circulating hormones [120]. (3) A load-dependent increase in cellular volume, enzymatic activities, and active electrolyte transport have been observed in the distal convoluted tubule, in experimental conditions in which the function of the preceding segment was blocked and the hormonal environment maintained constant [24, 121, 122]. These observations suggest that changes in luminal delivery might be the major cause of the protein- and ADH-induced increases in TAL function.

As explained earlier, the changes induced by high urinary concentrating activity or by high protein intake do not concern the

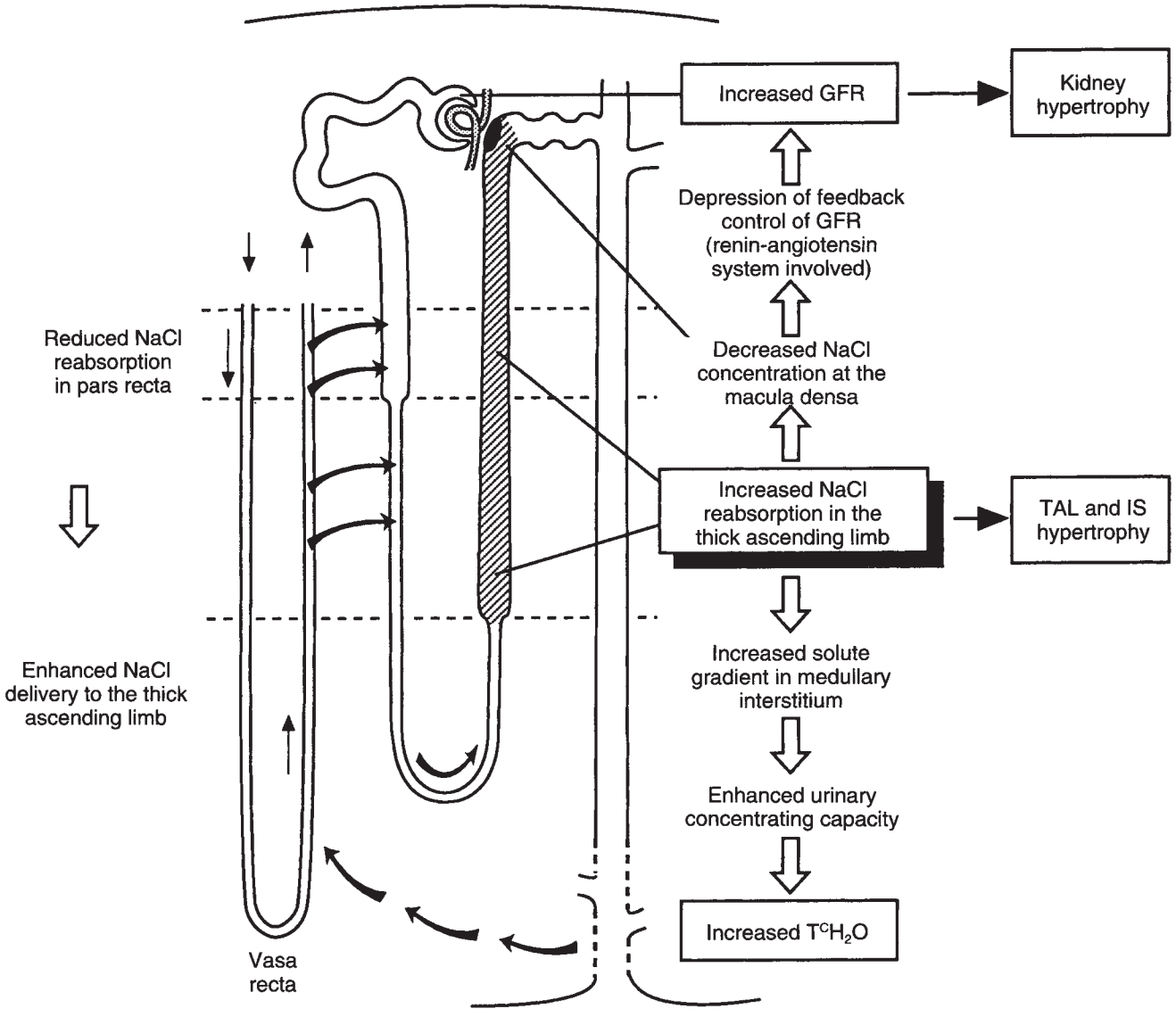


**Fig. 9.** *Proposed sequence of event explaining the changes seen in renal anatomy and function after stimulation of urinary concentrating activity or high protein intake.* The first, and well demonstrated effect in both situations is the hypertrophy and increased transport activity of the medullary thick ascending limb (MTAL). These changes can adequately explain the simultaneous increase in GFR and enhanced urinary concentrating capacity (see legends on the right part of the diagram). In contrast, the factor(s) which might initiate the increase in MTAL function and size are not yet well established. A decrease in water and sodium chloride reabsorption in the pars recta could increase solute delivery to the ascending branch of the loop and thus induce a load-dependent increase in MTAL transport (see legends on the left part of the diagram). This reduction in proximal reabsorption could result from the addition of urea into the lumen of the pars recta (dark arrows) due, for the concentration-(ADH)-dependent effect, to urea recycling, and for the protein-dependent effect, to the (probably indirect) action of glucagon on the pars recta (see text).

entire TAL uniformly. They are restricted to the medullary TAL and are most pronounced in the earliest part of this segment. Since, however, both the medullary and the cortical portions of the TAL exhibit ADH- and glucagon-sensitive adenylate cyclase and sodium transport [28, 112, 123], the unique pattern of TAL hypertrophy clearly points to a load-dependent process rather than to a hormonally-mediated influence.

### *Increased solute delivery to the TAL*

If an increased load to the MTAL seems the most probable cause of the increased function and hypertrophy of this segment, the next, yet unresolved question concerns the mechanism by which this load is increased. Studies in humans have shown that proximal reabsorption is reduced, and thus delivery to the loop of Henle increased after a protein meal [124, 125]. Could proximal reabsorption be decreased and sodium chloride delivery to the TAL increased in a similar fashion in response to either protein intake or stimulation of urinary concentrating processes by ADH? What could be the mechanism responsible for the diminished proximal reabsorption in each situation?

In the case of a protein meal, glucagon could be involved since

this hormone has recently been shown to reduce proximal reabsorption [99, 126]. Because glucagon does not stimulate adenylate cyclase activity in the proximal tubule and does not seem to possess specific receptors in this nephron segment [127, 128], its effect on the proximal tubule is probably indirect and could result from glucagon's prior action on the liver. In this organ, glucagon stimulates cAMP synthesis and release into the circulation [129–132]. In the kidney, in addition to being filtered, part of this cAMP is secreted by the organic acid transport system of the proximal tubule [133]. This nucleotide has been shown to compete with PAH for accumulation in proximal tubule suspensions [134, 135]. The resulting increase in intracellular concentration of cAMP seems responsible for the PTH-like effects of glucagon [114, 136]. The fact that the decrease in proximal reabsorption is not rapidly reversible upon cessation of glucagon infusion in rats (M. Ahloulay and L. Bankir, unpublished results) and is mimicked by an i.v. infusion of exogenous cAMP speaks in favor of this indirect mechanism [137] (see discussion in [99]).

In the case of the stimulation of urinary concentration, a reduction in proximal reabsorption has not been documented to our knowledge. However, several factors which could possibly be thought to induce a fall in proximal reabsorption are indeed modified during sustained high urinary concentration. First, a 60% increase in plasma cAMP concentration has been reported in normal subjects and patients receiving an infusion of dDAVP [138]. Although the origin of this increase is not yet well understood, it might increase secretion of cAMP in proximal tubular cells and thus induce a PTH-like effect, as explained above for glucagon.

Second, vasopressin or dDAVP favor an intense urea recycling. It is usually considered that the reintroduction of recycled urea from the ascending vasa recta to the descending branch of the loop takes place in the thin descending limbs of short looped-nephrons [59, 139] which, at least in rodents, run close to or within the vascular bundles in the inner stripe of the outer medulla [140, 141] and exhibit a relatively high permeability to urea [142]. Although less often mentioned, it is likely that additional urea could also be reintroduced upstream in the nephron, that is, in the medullary portion of the pars recta, in the outer stripe. This assumption is justified by the vascular-tubular relationships prevailing in this area. Medullary pars recta are surrounded by large, fenestrated vasa recta ascending from deeper regions of the medulla, and thus heavily loaded with urea [16, 141, 143, 144]. Most likely, a significant concentration gradient favors the diffusion of urea from ascending vasa recta to descending pars recta. Passive [145, 146] or active [147] urea secretion was observed in the isolated perfused rabbit pars recta. More recently, some evidence for urea secretion has been obtained in dog and humans by the use of pharmacological agents [148–150]. As suggested by the study of Mudge, Foulks and Gilman [151], the addition of urea in the proximal tubule lumen could result in an equivalent reduction of net fluid and solute reabsorption by an osmotic effect, as does the addition of secreted organic acids [152].

In summary, an enhanced secretion of recycled urea in the pars recta (in the case of vasopressin), or a glucagon (liver)-dependent increase in intracellular cAMP (in the case of proteins) could reduce proximal reabsorption and thus increase delivery of fluid and solutes to the thick ascending limbs, the common event thought to be involved in the concentration- and protein-dependent changes in GFR and kidney hypertrophy (Fig. 9).

## Functional consequences in health and disease

Because absolute urinary osmolality declines in CRF, it is generally considered that concentrating ability is impaired. However, since overall renal function is also impaired, the concentrating ability of the kidney in CRF is maintained as well as or even better than the filtration rate when evaluated as solute-free water reabsorption per unit GFR [153–155]. In a population of 116 outpatients with various primary renal diseases, we observed that, contrary to the usual belief, urine osmolality declines only slowly with progression of CRF. Average daily urine osmolality remained hypertonic over most of the progression until creatinine clearance fell to only ≈25 ml/min, demonstrating far from negligeable residual concentrating activity. On the other hand, vasopressin tends to increase with declining renal function [156, 157].

As reviewed in the first section, vasopressin and the stimulation of urinary concentrating activity tend to increase GFR. Since this concentrating capacity remains significant in the diseased kidney, and since vasopressin plasma level tends to increase, vasopressin probably contributes to inducing compensatory hyperfiltration in remnant nephrons or, when their individual filtration has reached its maximum, to unnecessarily enhancing glomerular pressure, thus accelerating the deterioration of these nephrons.

Given the effectiveness of a reduction in protein intake in slowing the progression of chronic renal failure (CRF), since, as reviewed above, dietary proteins influence renal function and morphology by stimulating the concentrating activity of the kidney, and since this concentrating activity alone tends to increase GFR, it is logical to asssume that a reduction in the concentrating activity by itself, that is, independent of any change in protein intake, could also be beneficial in slowing progression. This is indeed confirmed or strongly suggested by several observations.

(1) In Sprague Dawley rats with 5/6 nephrectomy, Bouby et al reduced urinary concentrating activity by increasing water intake (mixing their food with a water-rich agar gel). As expected, urinary osmolality and plasma vasopressin concentration fell in these rats. Proteinuria, systemic blood pressure (Fig. 10), kidney hypertrophy, incidence of glomerulosclerosis, and mortality [3] were all significantly reduced by this protocol, thus suggesting that the activity developed by the kidney for concentrating urinary solutes contributes to the progressive deterioration of remnant nephrons. Conversely, proteinuria and mortality were both agravated in 5/6 nephrectomized Brattleboro DI rats when they received a constant infusion of the antidiuretic, non-pressor vasopressin analogue dDAVP [158]. In addition, Brattleboro rats with DI show significantly less hyperfiltration in remnant nephrons than do rats without DI [159].

(2) Chronic renal failure is known to be a major cause of death in laboratory rats. These rats eat dry pellets throughout their lifetimes. Several studies have shown that, on a dry food regimen, water intake, although higher than on a more hydrated regimen, does not entirely compensate for the low water content of the food because of a shift in the thirst threshold [160, 161]. It is thus conceivable that chronic stimulation of urinary concentrating activity, imposing sustained hyperfiltration throughout life, contributes to the high incidence of CRF in aging laboratory rats. Actually, one report suggests that longevity is increased in rats fed hydrated diets [162].

(3) A high protein intake was reported not to aggravate

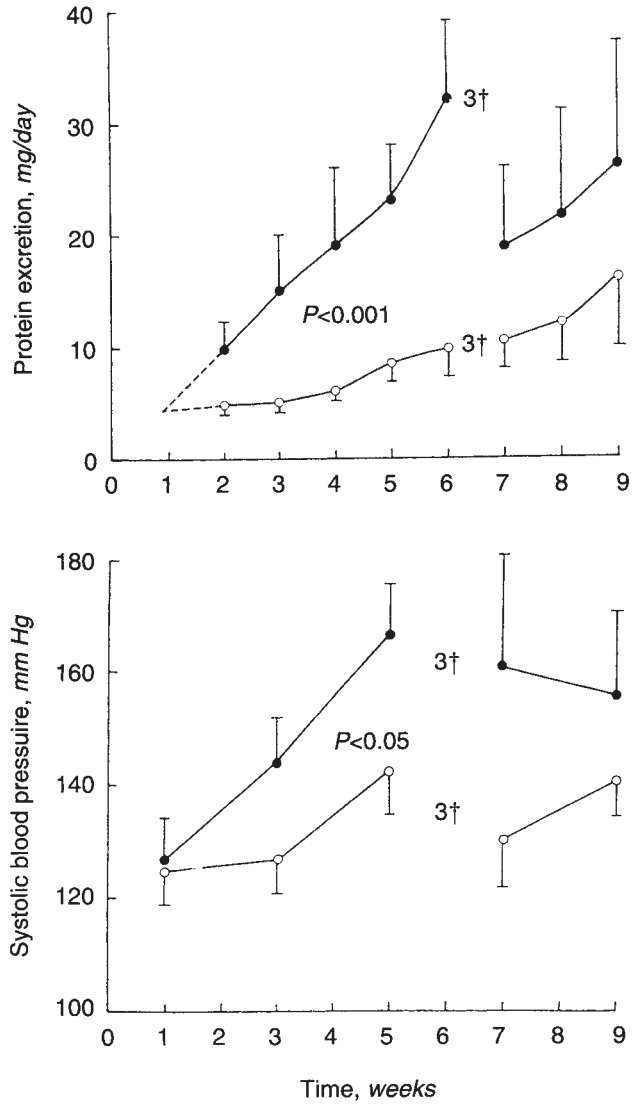


**Fig. 10.** *Influence of urinary concentrating activity on progression of chronic renal failure.* Sprague-Dawley rats with 5/6 nephrectomy were given for 10 weeks an increased water intake by mixing their food with a water-rich agar gel (○, HWI) and were compared to rats with similar initial renal impairment but normal water intake (●, NWI). The time-dependent increases in both proteinuria and blood pressure were markedly reduced by the reduction of the urinary concentrating activity (average urinary osmolality was 390 ± 9 in HWI vs 946 ± 122 in NWI). On the sixth week of the study, 3 NWI rats (of the initial 9) died from advanced renal failure and the 3 corresponding HWI rats were sacrified (shown by 3†). Adapted from [3]; used with permission.

progression in rats with lithium-induced CRF [163]. This protection might be explained by the fact that lithium inhibits the action of ADH on the collecting ducts [164] and thus compromises urinary concentrating capacity and vasopressin-dependent urea recycling.

(4) It has recently been shown that parathyroidectomy reverses the deleterious effects of high protein intake in rats with reduced renal mass [165]. Although not discussed by the authors, it is logical to assume that the protective effect of parathyroidectomy could be linked to the significant potentiation of urinary concentration by PTH [166]. Although urinary flow rate and/or osmolality were not available, the marked reduction in kidney weight seen in parathyroidectomized rats [165] is reminiscent of the low kidney weight observed in animals in which urinary concentration is impaired (Tables 1 and 3) [2, 158].

(5) The last piece of evidence supporting the role of urinary concentration in progression concerns ammonia. Ammonia, one of the end products of protein metabolism is very toxic and its plasma level remains very low (≈50 μmol/liter). Thus, an efficient system is required for enabling its concentration in the urine. This concentration is achieved by an active secretion and a complex medullary recycling that prevents ammonia, synthesized and secreted in the proximal tubules, to leak from the cortical parts of the distal nephron. Interestingly, Nath, Hostetter and Hostetter observed a marked reduction of progression of CRF when ammonia excretion, and thus its urinary concentration, was reduced by chronic bicarbonate supplementation [167].

These observations in experimental models of CRF suggest that a chronic increase in fluid intake or the administration of specific vasopressin $V_2$ antagonists [168] could have a beneficial influence in patients during the early phase of the disease when they still produce hypertonic urine [156]. The objective would be to reduce the natural tendency to concentrate urine so as to bring urine osmolality close to isotonicity. Excessive water intake would of course challenge the kidney with another undesirable burden, that of producing dilute urine in order to excrete unappropriate water loads.

In summary, convergent information suggests that the functional steps involved in concentrating solutes in the urine represent a burden for the diseased kidney. Reducing either the amount of solutes to be concentrated (by reducing protein intake) or reducing the concentrating effort (by providing more fluid for the excretion of an unchanged amount of solutes) are two independent and probably additive ways to minimize this burden.

Lise Bankir and Wilhelm Kriz
*Paris, France and Heidelberg, Germany*

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*Correspondence to Lise Bankir, INSERM Unité 90, Hôpital Necker, 75743 Paris Cédex 15, France, or Wilhelm Kriz, Institut für Anatomie und Zellbiologie der Universität Heidelberg, Im Neuenheimer feld 307, D-69120 Heidelberg, Germany.*

## Appendix. Abbreviations

| | |
|---|---|
| ADH | antiduretic hormone, vasopressin |
| AVP | arginine-vasopressin |
| CD | collecting duct |
| CRF | chronic renal failure |

| | |
|---|---|
| dDAVP | 1 deamino 8-D-arginine vasopressin |
| DI | diabetes insipidus |
| $FE_{urea}$ | fractional excretion of urea |
| GFR | glomerular filtration rate |
| IS | inner stripe of the outer medulla |
| MTAL, CTAL | medullary and cortical parts of the TAL |
| PPF | papillary plasma flow rate |
| SNGFR | single nephron glomerular filtration rate |
| TAL | thick ascending limb of Henle's loop |
| $T^cH_2O$ | free water reabsorption |
| TGF | tubuloglomerular feedback |

## Note added in proof

Several observations pertinent to the questions discussed in this review have become available recently. (1) Infusion of dDAVP (an antidiuretic analogue of ADH devoid of vascular action) has been shown to increase renal plasma flow in humans [169]. It is interesting to note that, in this study, subjects were not submitted to water diuresis prior to dDAVP infusion, thus probably preventing the volume expansion and/or plasma dilution induced by massive water intake as occurred in many previous studies. (2) The marked and preferential hypertrophy (and hyperplasia) of the MTAL observed in the hypertrophied kidney after high protein intake might be triggered by insulin-like growth factor I (IGF-1), a hormone for which a high density of receptors is selectively found in this nephron segment. The gene expression of IGF-1 receptor in the MTAL was markedly increased, and that of IGF binding protein (reducing IGF-1 availability for binding to its specific receptors) decreased after 2 to 7 days of high protein intake in rats [170]. However, as stated by the authors, the stimulus initiating these changes is still unknown. Intriguingly, in the same study, high protein intake in Brattleboro rats with DI (40% vs. 18% casein diet for 4 days) was found to induce a normal hypertrophy of the kidney [170] in contrast to the results of Bouby et al (32% vs. 10% casein diet for 7 weeks) [102]. (3) Patients with diabetes insipidus were found to have a lower GRF than control subjects, as previously observed in Brattleboro DI rats. They, nevertheless, exhibited a normal rise a GFR after a protein meal [171].

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